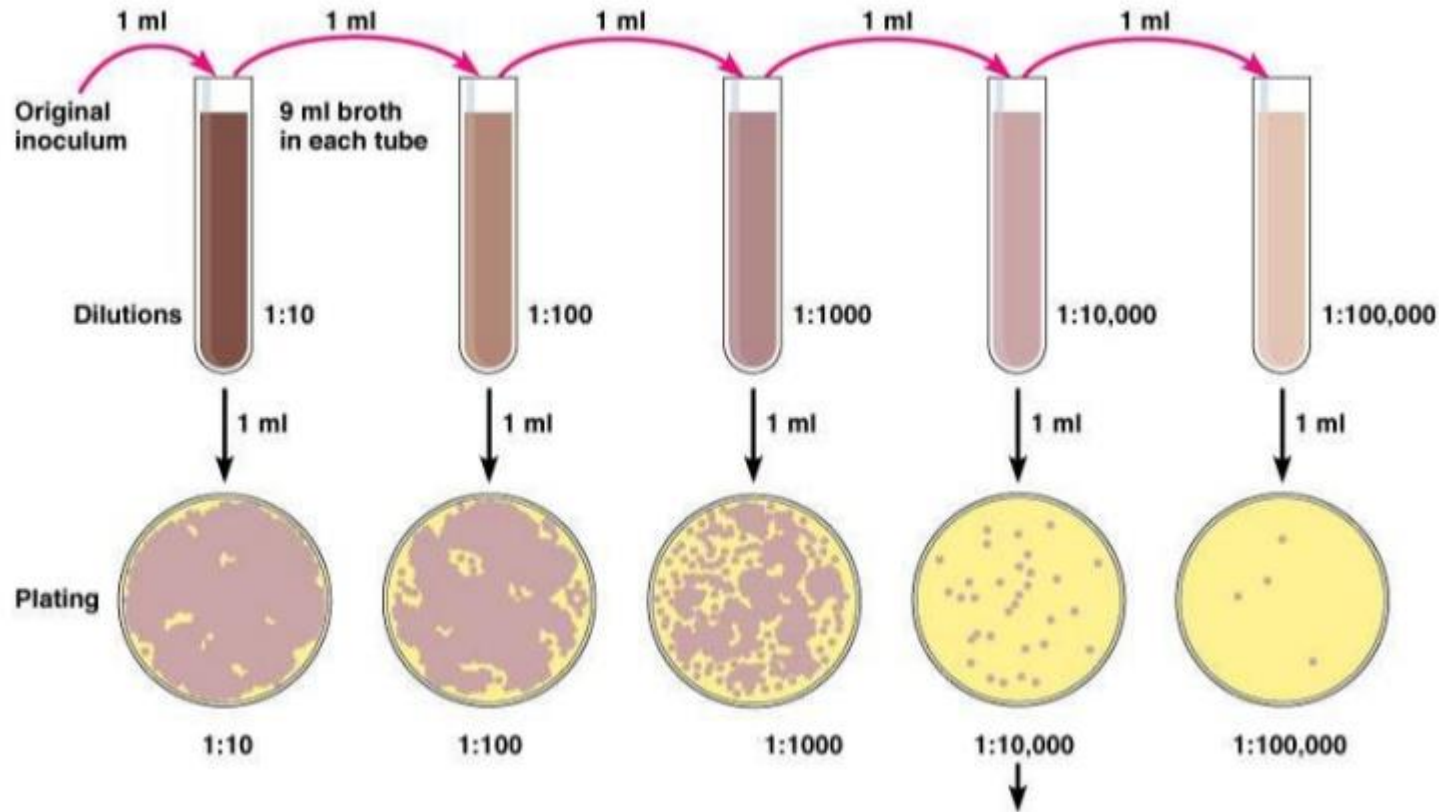


Dilution techniques

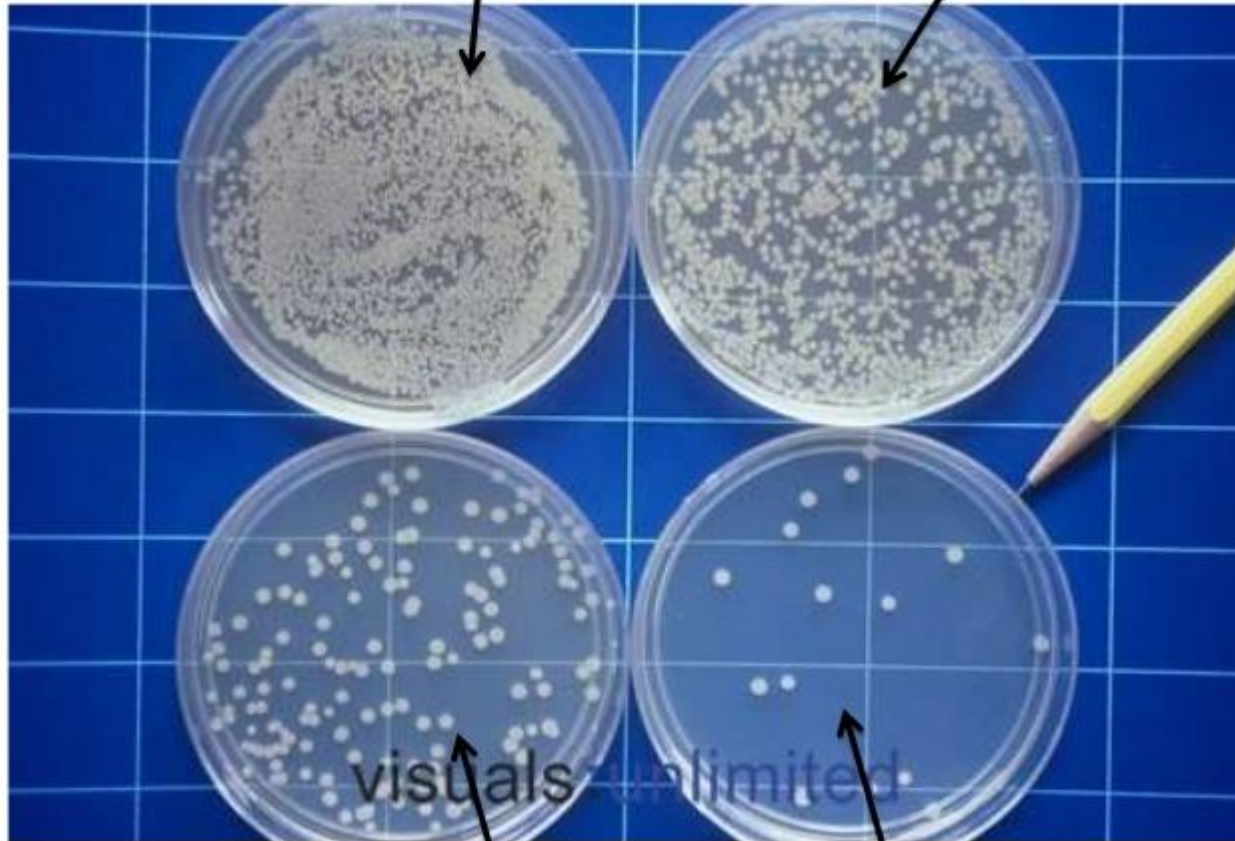
Serial Dilution



Calculation: Number of colonies on plate $\times$ reciprocal of dilution of sample = number of bacteria/ml
(For example, if 32 colonies are on a plate of $1/10,000$ dilution, then the count is $32 \times 10,000 = 320,000/\text{ml}$ in sample.)

Too much growth, hard to count colonies

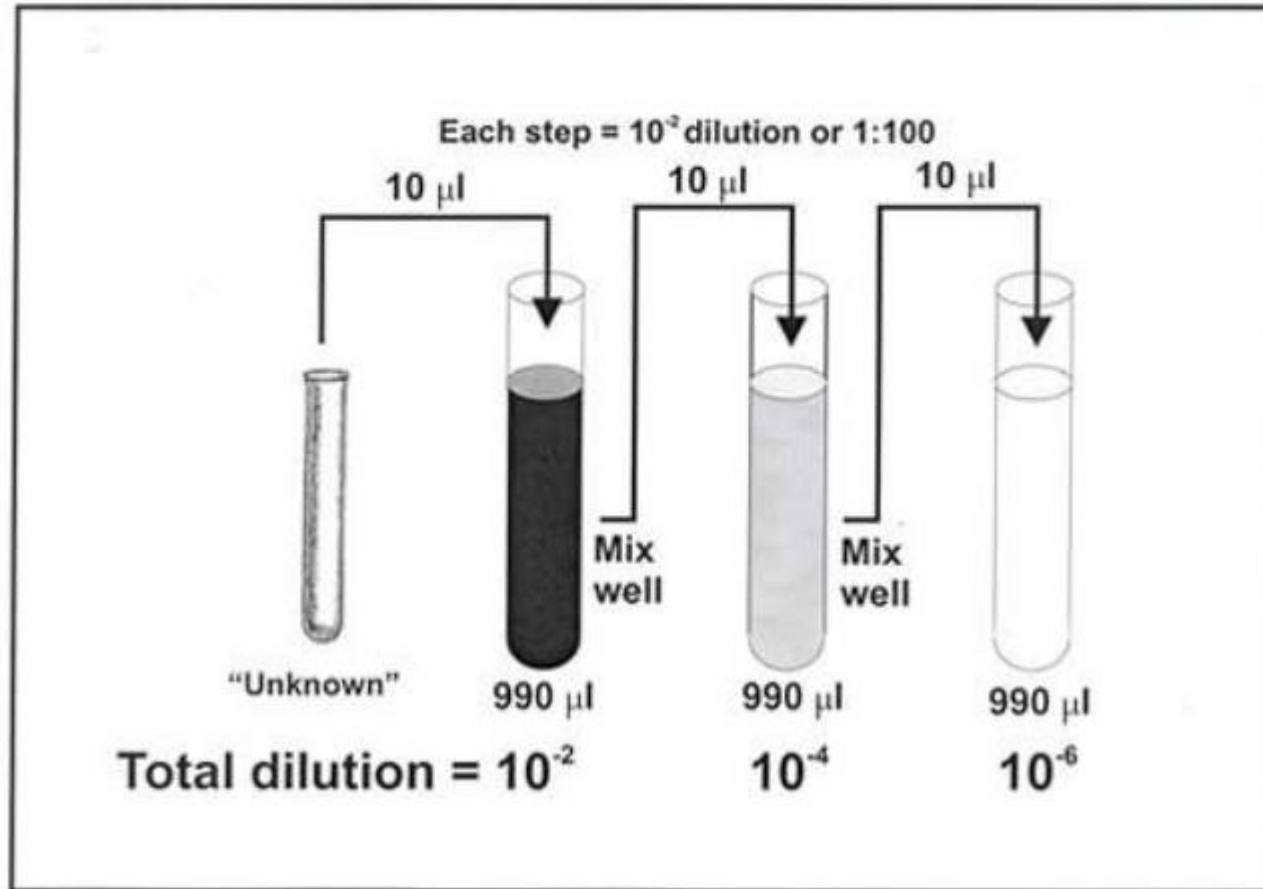
Growth not as much, but still hard to count colonies



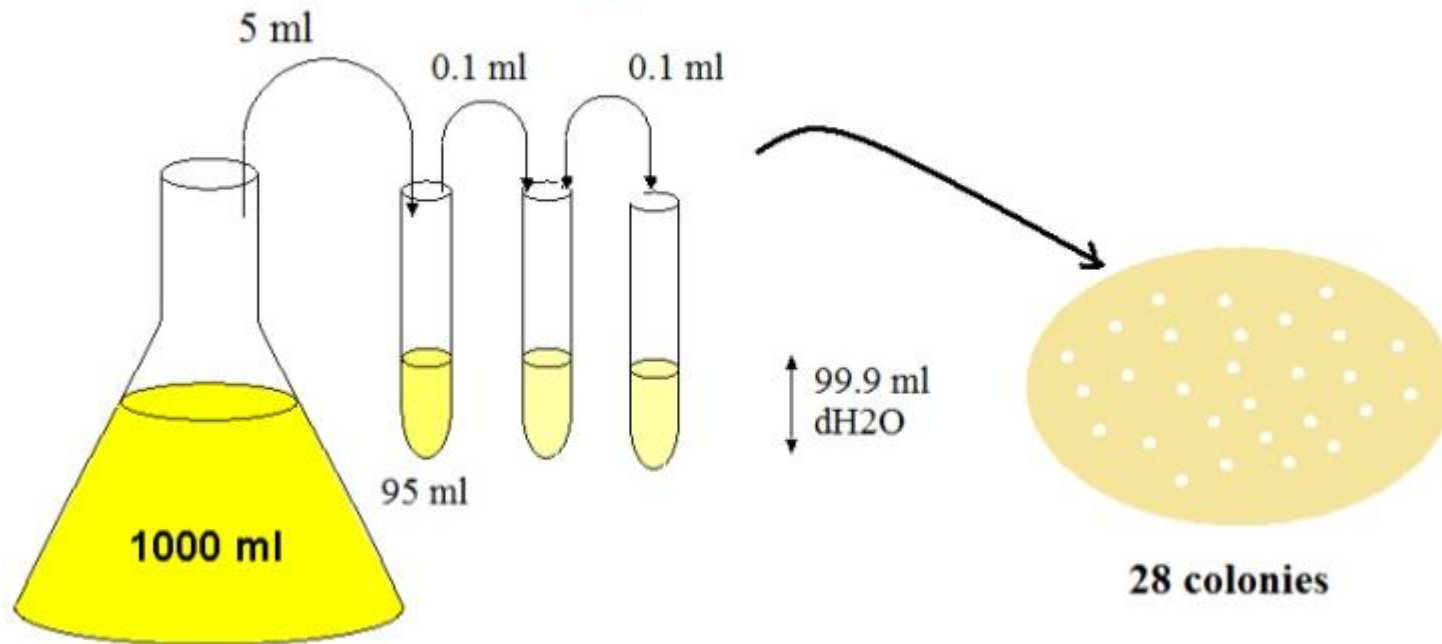
OK to count but there are some colonies that are not single colonies, they are so close together

Definitely the best plate to estimate the CFU

Serial Dilution cont'd



Try this!!!!



What is the CFU of the stock?

HOW TO CALCULATE CFU/ml IN A GIVEN SAMPLE?

A plate of the 10^6 dilution yielded a count of 130 colonies. Calculate the number of bacteria in 1 ml of the original sample?

CFU x Dilution factor

$130 \times 1000000 = 130,000,000$ or 1.3×10^8 CFU/ml of the original sample.

Five ml of Bacterial Culture is added to 45 ml of sterile diluent. From this suspension, two serial, 1/100 dilutions are made, and 0.1 ml is plated onto Plate Count Agar from the last dilution. After incubation, 137 colonies are counted on the plate. Calculate CFU/mL of the original Sample?

CFU x Dilution factor

Dilution factor: 5 ml into 45 ml = 1:10 1: 100 1:100 = 10^5 .

$137 \times 100,000 = 13700,000 / 0.1$ (volume of sample) = 137,000,000 or 1.37×10^8 CFU/ml of the original sample.

To convert it into log value: Just take Log(CFU/mL)

Here, $\log (1.37 \times 10^8) = 8.13924922$. □

Useful for expressing log reduction of microbes / biologic log reduction.

1. One ml of a bacterial culture was pipetted into a 9 ml dilution blank. One-tenth ml of this dilution was pipetted into a 9.9 ml dilution blank. From this dilution, one-tenth ml was plated with 25 ml of culture medium. 220 colonies arose after incubation. How many colony-forming units were present per ml of the original culture?

One ml into 9 ml dilution blank. 1:10

One-tenth ml of this dilution (1/10) or 0.1 ml was pipetted into a 9.9 ml dilution blank=1:100

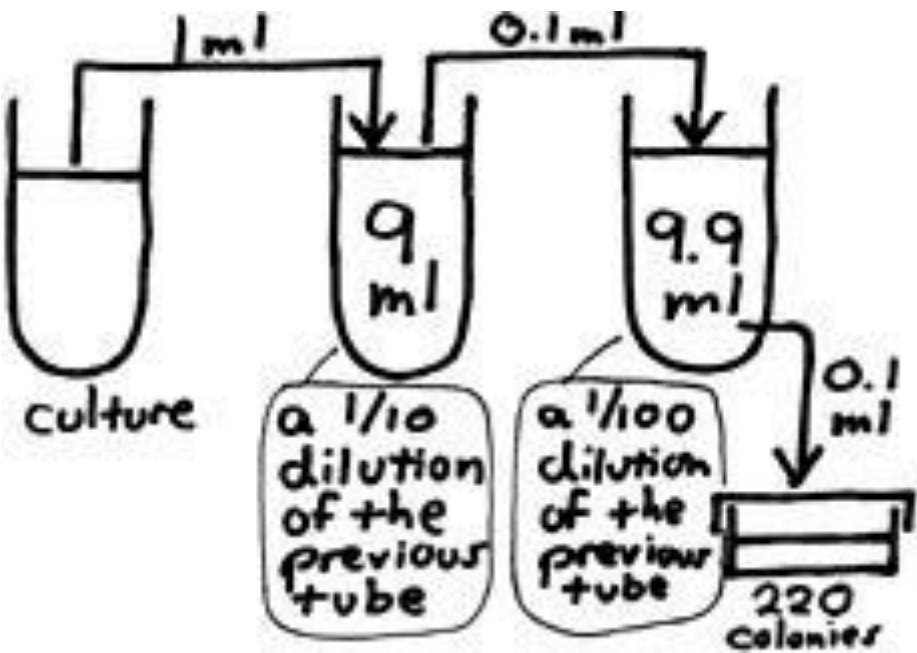
1: 10 and 1:100 =1:1000 dilution

From this dilution, one-tenth ml (0.1 ml) was plated with 25 ml of culture medium.

No. of Colonies= 220 colonies X dilution factor (10^4).

How many colony-forming units were present per ml of the original culture?

$220 \times 1000 \times 10 = 2200000$ or 2.2×10^6 .



Q. Ten ml of spring water were added to a petri dish to which 40 ml of melted Plate Count Agar were added. After incubation, 35 colonies arose on the plate. What was the count of CFUs per ml of the spring water?

Ans. Ten ml of spring water were added to a petri dish= 3.5 colonies or CFU /ml of the spring water.